A METHOD OF ISOLATING ANTHRAX BACILLI FROM THE SOIL

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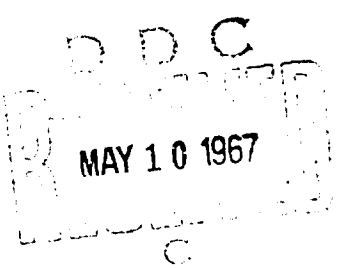

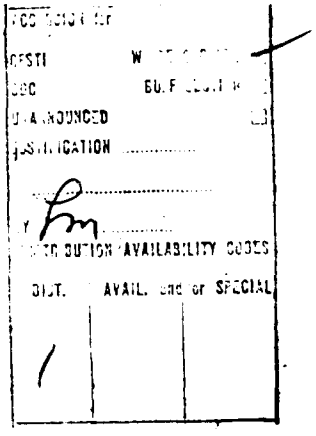

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A METHOD OF ISOLATING ANTHRAX BACILLI FROM THE SOIL

[Following is the translation of an article by M. F. Shapovalova, Krasnodar Regional Sanitary-Epidemiological Station, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), 1966, Vol 43, No 1, pp 144-146. It was submitted on 14 Apr 64. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

In the Soviet Union human incidence with anthrax has been sharply reduced thanks to the carrying out of combined veterinary and sanitary measures.

One of the reasons for the extensive and persistent anthrax incidence in individual territories is the ability of the causative agent to form spores, the viability of which is exceedingly high. Shlyakhov (1957, 1960), Terentyev (1956), and Mikhin (1942) assign particular importance to soil foci -- territories which many years ago were inseminated with the spores of the anthrax bacilli. A number of authors demonstrated that the soil is not only a site for the prolonged preservation of anthrax spores, but it is also a substrate in which under a specific humidity, temperature and pH, vegetation takes place, that is the spores germinate. Soil infected with anthrax microbes remains an enzootic focus for a long time, since up to the present time there are not sufficiently effective methods for rendering infected soils harmless.

In the Krasnodar Kray 1402 fixed points have been recorded which are unsafe for anthrax; these have been studied since 1944. The greatest number of foci have been counted in Timashevskiy, Kavkaskiy, N. Pokrovskiy, Dinskiy, and Kushchevskiy Rayons, However, in spite of the threatening epizootological situation, caused by the presence of a large number of abandoned cattle burial grounds, the burial of cattle in undetermined places and the appearance of new foci, all the same a lowering has been noted in human cases of anthrax in the Kray.

From 1958 through 1963 we investigated 87 samples of soil, taken from foci in 25 rayons of the Kray (based on the old administrative divisions). The soil samples were taken in a sterile glass container.

The isolation of the anthrax causative agent was performed simultaneously by two methods: Based on Dolda, and by the bacteriological and biological method developed by us.

When carrying out the method according to Dolda the soil was drenched with a physiological solution, then a surplus of crystalline urea was added so that on the bottom of the test tube there remained a small amount of undissolved urea and the mixture was left for 30 minutes at 37°. After treatment with urea the residue was seeded on 3--5 dishes with meat-peptone agar. The seedings were examined after 18--24 hours.

The essence of the method developed by us is as follows: 100-200 grams of soil is wet down with a double volume of physiological solution, mixed well, and then the suspension is allowed to remain for 1--2 hours; using a hyperdermic or a Pasteur pipette we took 3--4 ml of the supernatant liquid (on the border of the soil residue with the liquid) for investigation and divided it into two equal parts. From one of the parts an inoculation was made on 2--3 dishes with meat-peptone agar by the transfer method, the remainder was used to infect laboratory animals -- white mice and guinea pigs. The other portion of the liquid was preliminarily heated at 70° for 30 minutes, after which the investigation was carried out by the same plan pointed out above. After 18--24 hours we examined the seedings. The colonies which were suspect of anthrax were selected and with a washing from this culture we infected laboratory animals. The meat-peptone broth (with the heated portion of the suspension) was reseeded in dishes with meat-peptone agar. The culture obtained was used to infect white mice and guinea pigs. The infected animals were observed for a period of 10 days.

The internal organs of the dead laboratory animals (spleen, liver, lungs), blood from the heart, and the inguinal lymph nodes were seeded in dishes with meat-peptone agar, in test tubes with meat-peptone broth, and in dishes with meat-peptone agar and 5% sheep blood. From the blood and the internal organs of the animals we prepared smear-imprints which were stained by the generally accepted method. The precipitation reaction according to Ascoli was set up with the extracts from the organs of the dead laboratory animals.

As a result of the analyses which were carried out we isolated 8 anthrax cultures from the nonheated soil suspension. Of these, 7 were obtained by the biological and one by the bacteriological method.

The isolated cultures were identified as Bac. anthracis on the basis of the following features: They were all non-motile and produced a capsule in a living organism. Cultures on meat-peptone agar and in meat-peptone broth had the characteristic appearance, in the dishes with the blood meat-peptone agar not one culture lyzed erythrocytes. During seeding by injection in a gelatin column growth was observed in the form of a fir tree, turned with the top downward. The cultures curdled and then peptonized milk; the Ascoli reaction with antigen from the organs of the dead laboratory animals (from which the anthrax causative agent was isolated) was positive in all cases.

It is necessary to note that the periods of death of the infected animals were various: One white mouse (Tuapsinskiy Rayon) died after 120 hours, one guinea pig (Sovetskiy Rayon) -- after 96 hours, one guinea pig and 2 white mice (Psebayskiy and Anapskiy Rayons) -- after 72 hours, and 3 white mice (Otradnenskiy, Dinskoy and Kavkazskiy Rayons) -- after 48 hours. In the dead animals in the majority of cases the typical pathologoanatomical picture for anthrax was observed: Serous-jelly-like infiltration of the subcutaneous tissue, non-coagulated blood, and spleen filled with blood.

When investigating the same portions of soil by the method of Dolda the anthrax causative agent was not isolated in one case. In the seedings of soil which were treated with urea a sparse growth of soil saprophytes was noted on meat-peptone agar.

We investigated 40 anthrax foci. Positive results were obtained during the investigation of soils taken from foci in 7 Rayons of the Kray (Tuapsinskiy, Psebayskiy, Sovetskiy, Otradnenskiy, Dinskoy, Anapskiy and Kavkazskiy), where incidence was recorded among horned cattle and small cattle on the collectives and in individual sectors. The maximum of unsafe points (58--67) were recorded in the Kavkazskiy and Timashevskiy Rayons. In 1963 anthrax epizootics were also noted in 6 of the stated rayons. In 4 of these (Psebavskiy, Sovetskiy, Otradnenskiy, Dinskoy) these were among animals in the collective and state farm sectors, and in 2 (Tuapsinskiy and Kavkazskiy) -- among cattle from individual sectors. In 5 rayons anthrax incidence was noted among humans.

The soil samples from the foci of the Sovetskiy, Otradnenskiy, Psebayskiy, Anapskiy and Kavkazskiy Rayons were taken in June--August, and from the foci of Tuapsinskiy and Kinskiy Rayons - in the spring time (end of March and the end of April).

The isolation of anthrax cultures was of particular interest in two cases. In Tuapsinskiy Rayon the source of an epizootic among cattle in an individual sector was exposed. The anthrax causative agent was isolated from soil samples taken in a pasture in the village of Tengink. In March 1958 a resident of this village fell sick with anthrax. The source of infection was a cow belonging to the sick person. A culture of the anthrax causative agent was isolated from the organs of a white mouse and a guinea pig infected with an unheated suspension of soil taken in the pastures. Urgent measures were taken for rendering the focus harmless: The area of the pastures was plowed over and a fruit garden planted, and the entire population of the village was inoculated with the STI vaccine.

In August 1963 in Kavkazskiy Rayon a resident from Kazanskaya village who had performed the autopsy of a dead sheep fell ill with anthrax. After disinfection was carried out in the focus a sample of soil was taken and from this sample the anthrax causative agent was isolated by the biological method. On our request veterinary workers again disinfected the focus.

In Anapskiy Rayon in 1959--1962 an anthrax epizootic was recorded among cattle in an individual sector of the "Pervomayskiy" state farm. During a planned investigation of the Rayon in June 1963 soil samples were taken in the cattle burial ground of the Chekhon farmstead. These were taken from the surface and at a depth of 10 cm. Using the biological method, the anthrax causative agent was isolated from the soil in both cases.

In the remaining 4 rayons the anthrax microbe was obtained from soil samples taken from foci where in 1--3 weeks prior anthrax epizootics were exposed among cattle on the collective and state farms.

Conclusion

The proposed method for isolating the anthrax causative agent from the soil is practicable and more effective than Dolda method.

Literature

Mikhin, N. A., Anthrax of man and farm animals, Moscow, 1942.

Terentyev, F.A., Anthrax of animals and how to combat it, Moscow, 1946.

Shlyakhov, E.N. Zh. mikrobiol., 1957, No 11, p 137.

Idem., Epidemiology, diagnosis and prophylaxis of anthrax, Kishinev, 1960.